Literature report

Allenamides as Orthogonal Handles for Selective Modification of Cysteine in Peptides and Proteins**

Ata Abbas, Bengang Xing,* and Teck-Peng Loh*

Qian Wu
2014.8.21

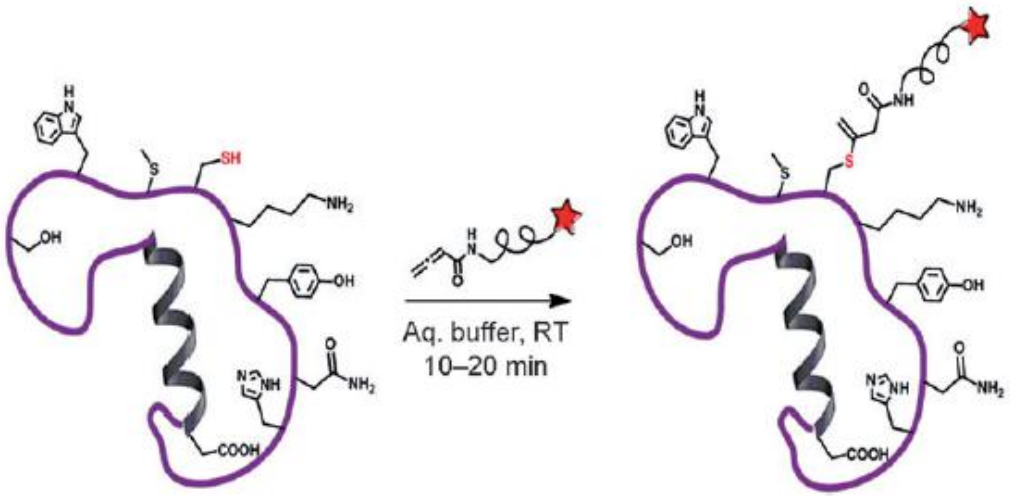
Angewandte International Edition Chemie

Protein Modifications

DOI: 10.1002/anie.201403121

Allenamides as Orthogonal Handles for Selective Modification of Cysteine in Peptides and Proteins**

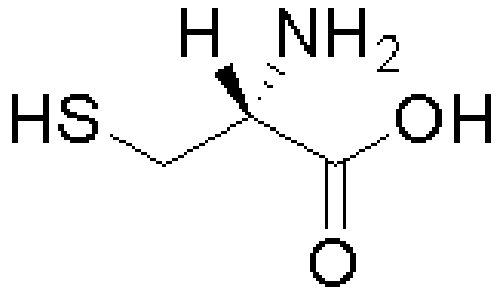
Ata Abbas, Bengang Xing, and Teck-Peng Loh**



Orthogonal handle
Simple and direct
Selectively
Quantitative conversion
Stable and irreversible
High reaction rates

Backgroud

Selective chemical modification of protein:

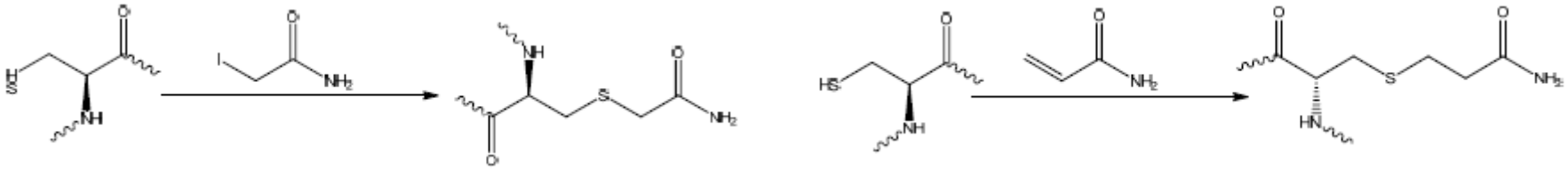


Higher nucleophilicity
Lower natural abundance

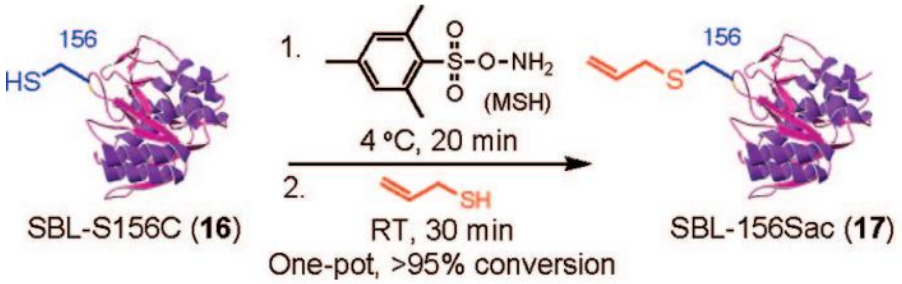
The sulfhydryl group in peptides and proteins has remained an attractive target for site-selective modification.

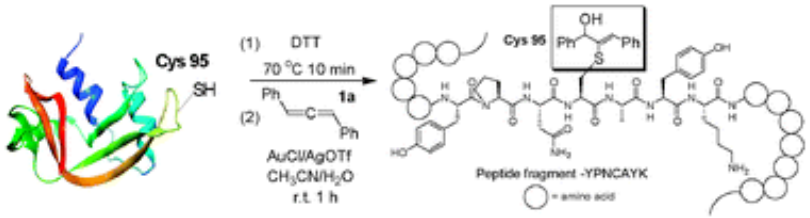
Two typical chemical pathways:

1. Nucleophilic substitution or Michael addtion



2. Metal-catalyzed Cys modification





B. G.Davis, *J. Am. Chem. Soc.* **2008**

C.-M. Che*, Chem. Commun*. **2013**

- However

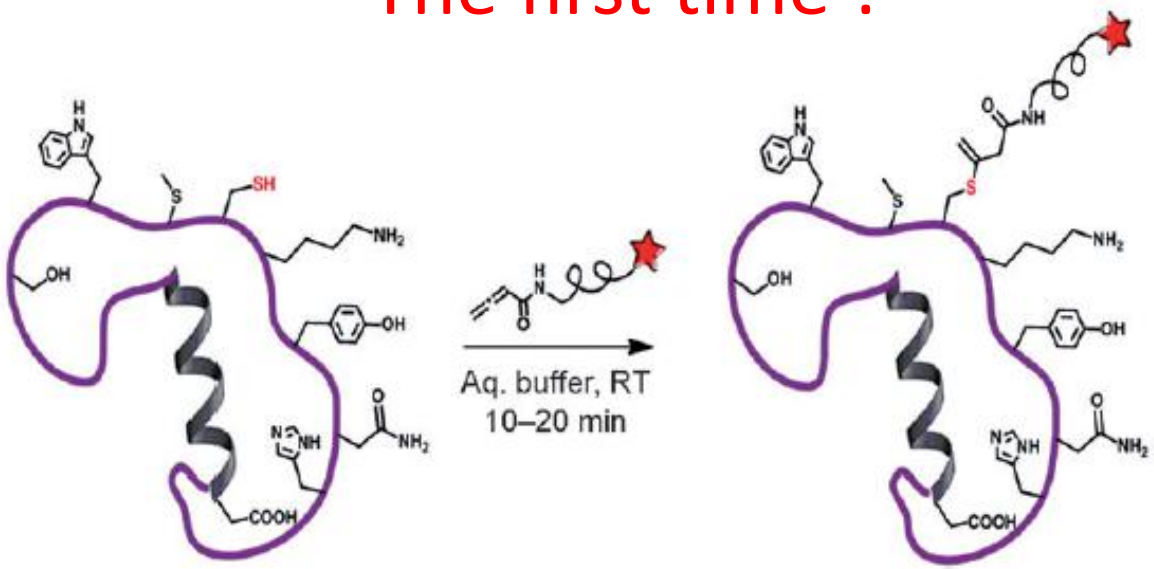
1. Biocompatibility
2. Selectivity: histidine and lysine residues
3. Reversibility/irreversibility: DTT and GSH

- Therefore

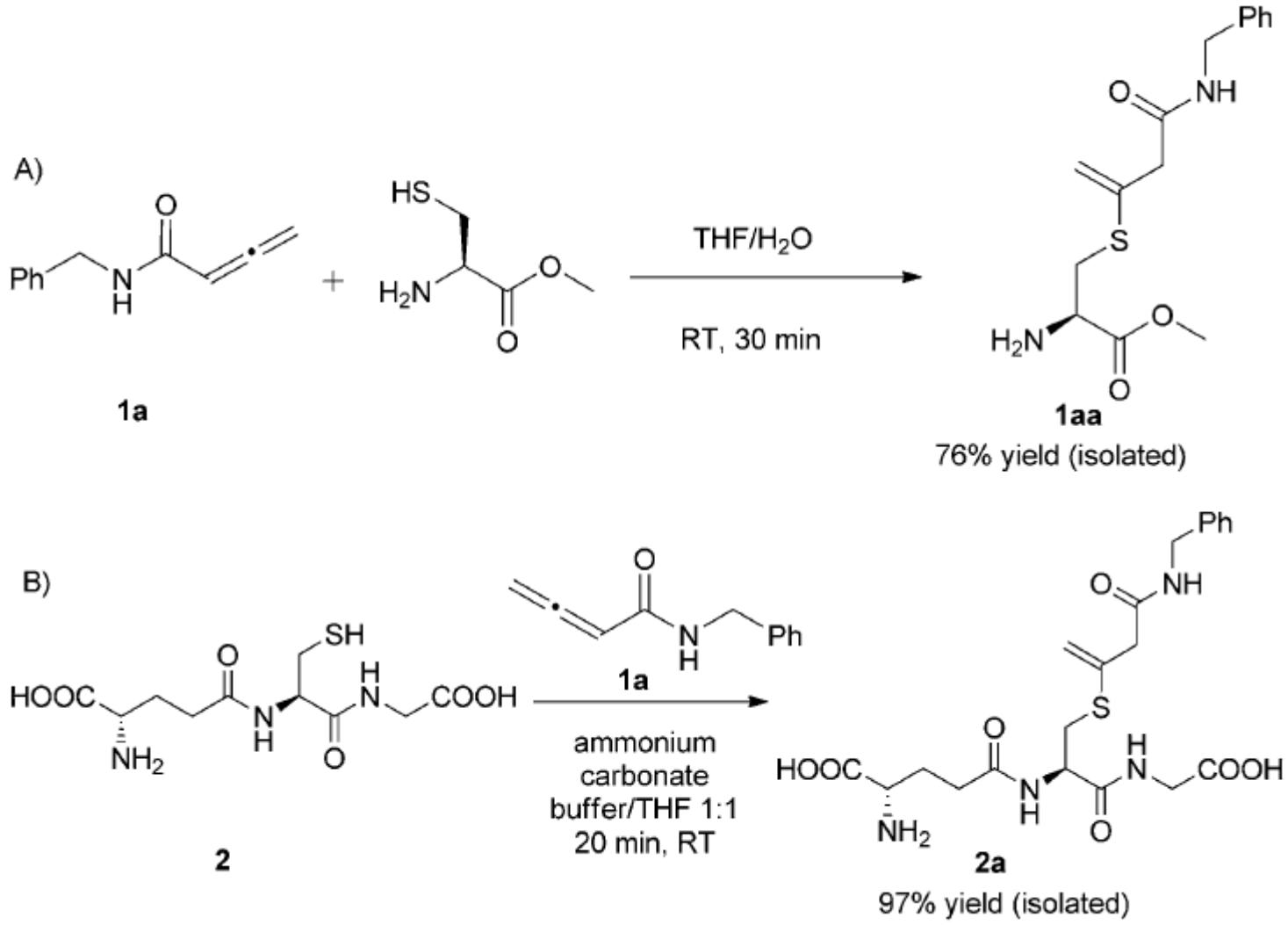
An urgent need exists to find promising orthogonal handles and related labeling strategies which can selectively and irreversibly bind with cystein.

C-substituted terminal allenamide moieties

The first time !



Orthogonal handle
Selectively: no reaction with –OH,-NH_2,-COOH
Quantitative conversion
Stable and irreversible
High reaction rates: 10-20 min
Mild reaction condition: aqueous buffer(pH 8.0), r.t.
Easily prepared, stable at r.t.



C-substituted terminal allenamides showed the excellent reaction selectivity of with the specific cysteine conjugation instead of the amino groups.

Selectivity

- Peptide: Cys-Gly-Lys-Ser-Arg-Phe (3)

  Lys-Ser-Cys-Gly-Arg-Phe (4)

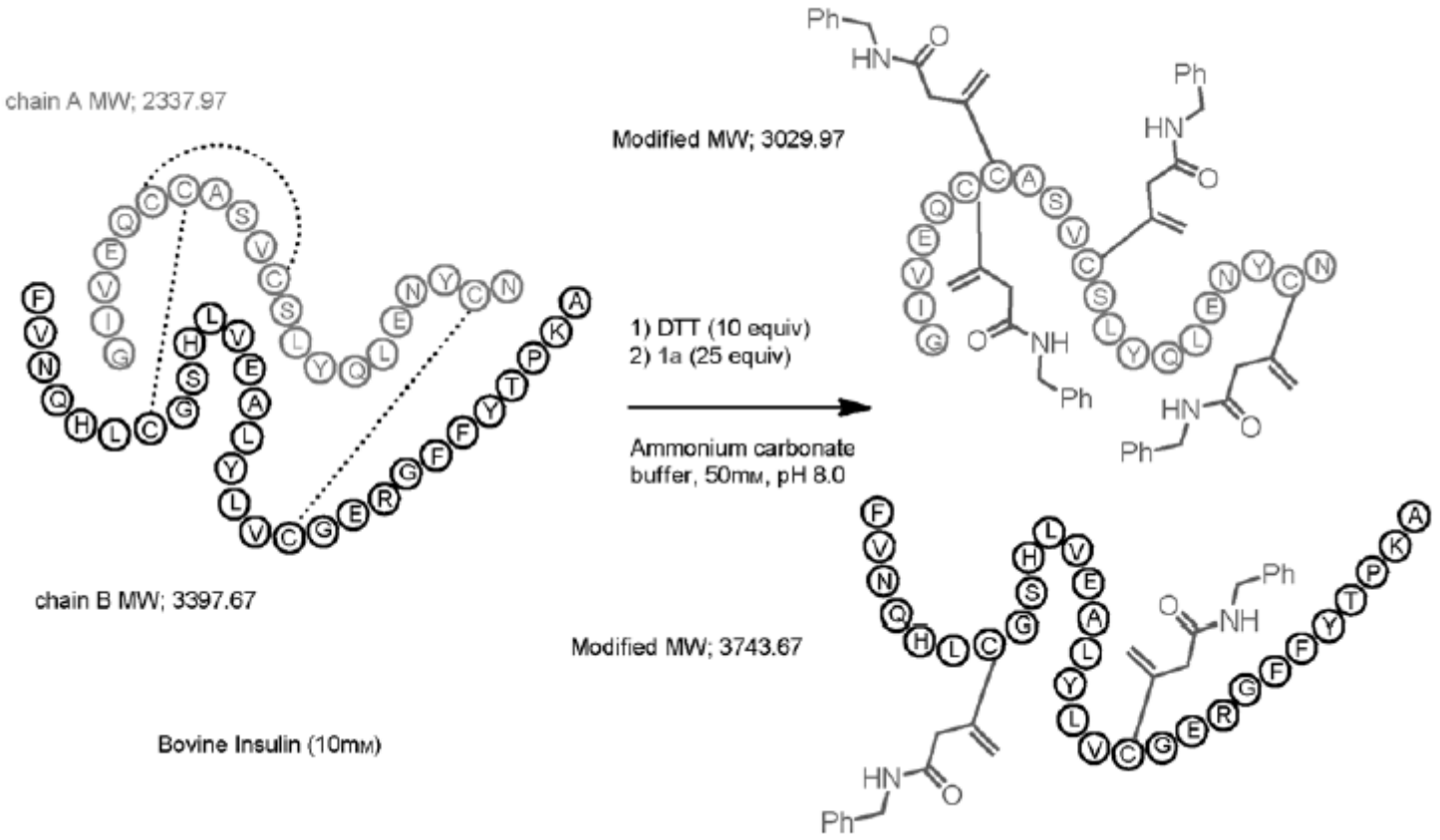
  Tyr-Asp-Ser-Gln-Cys-Phe-His-Arg-Trp (5)

peptide (250 mm) with 1a (10 equiv) for 10 minutes in ammonium carbonate buffer (pH 8.0) at r.t.

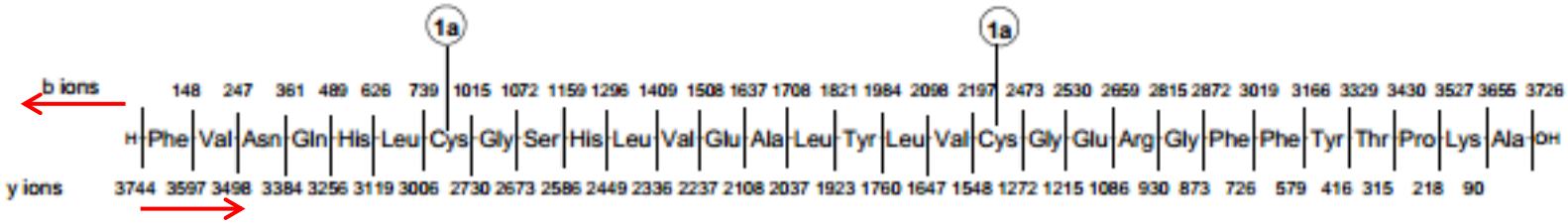
Irreversibility

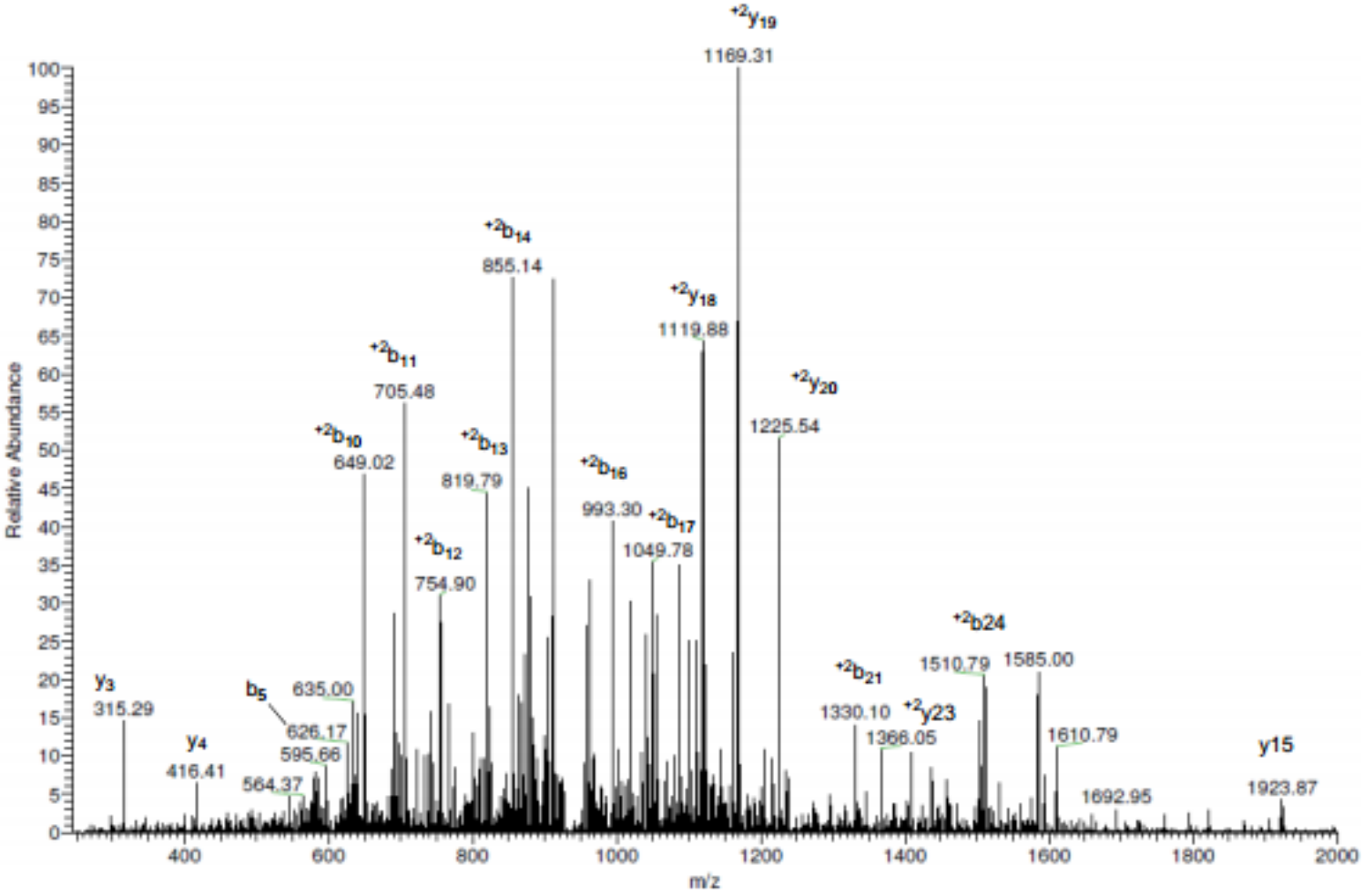
- Peptide 5 + 1a + excess (100 equivalent) GSH
  2a + excess (100 equivalents) DTT

The good irreversibility of specific cysteine labeling may thus enable the possibility for the protein modification under biological conditions.



A more complex pair of peptides, generated from the DTT treatment of bovine insulin, was treated with 1a to afford the fully modified chains A and B.


1a
1a
b ions
148 247 361 489 626 739 1015 1072 1159 1296 1409 1508 1637 1708 1821 1984 2098 2197 2473 2530 2659 2815 2872 3019 3166 3329 3430 3527 3655 3726
H Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Ala OH
y ions
3744 3597 3498 3384 3256 3119 3006 2730 2673 2586 2449 2336 2237 2108 2037 1923 1760 1647 1548 1272 1215 1086 930 873 726 579 416 315 218 90


Relative Abundance
m/z
+2y19
1169.31
+2b14
855.14
+2y18
1119.88
+2b11
705.48
+2y20
1225.54
+2b10
649.02
+2b13
819.79
+2b16
993.30
+2b17
1049.78
+2b12
754.90
+2b24
1510.79
1585.00
+2b21
1330.10
+2y23
1366.05
1610.79
y15
1923.87
1692.95
y3
315.29
b5
635.00
626.17
595.66
564.37
y4
416.41

MS/MS for the modification of 5 (YDSQCFHRW) with 1a

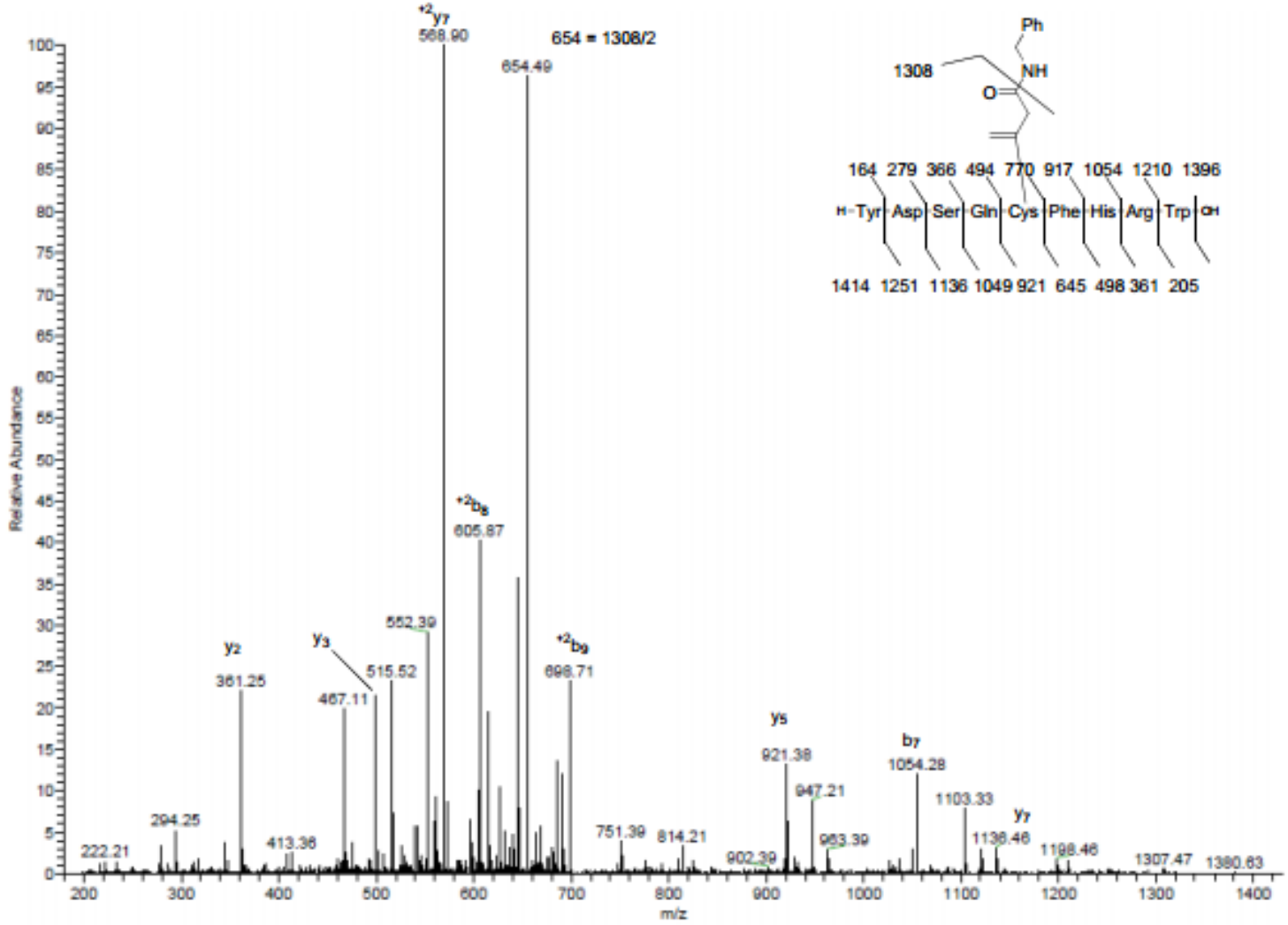
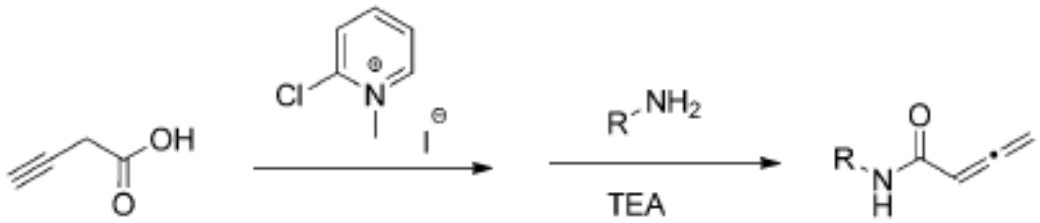
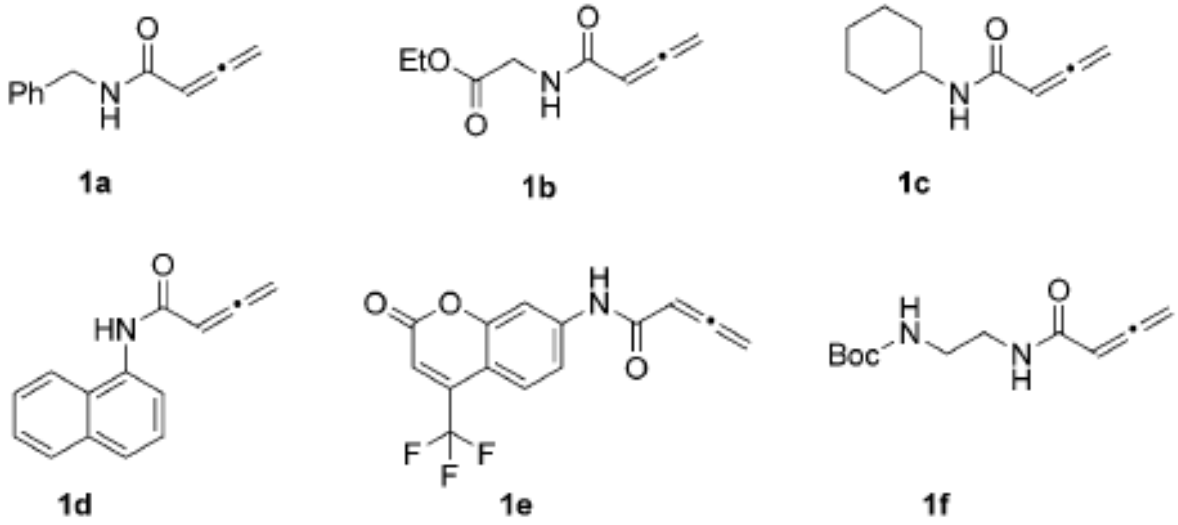


Figure 6 showing MS/MS of peptide YDSQCFHRW modified with 1a

Table 1: C-substituted allenamide synthesis from amines.[a]

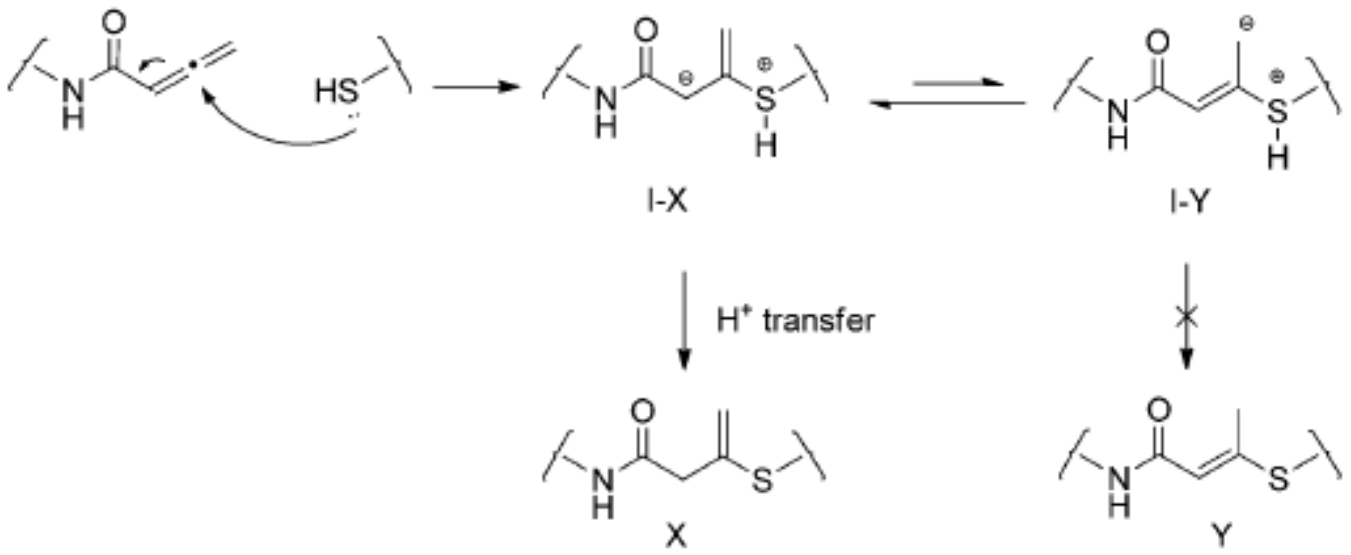




[a] A small amount of homopropargyl amide is also observed sometimes and can be isomerized to the allenic isomer by excess TEA. Boc — *tert*-butoxycarbonyl, TEA — triethylamine.

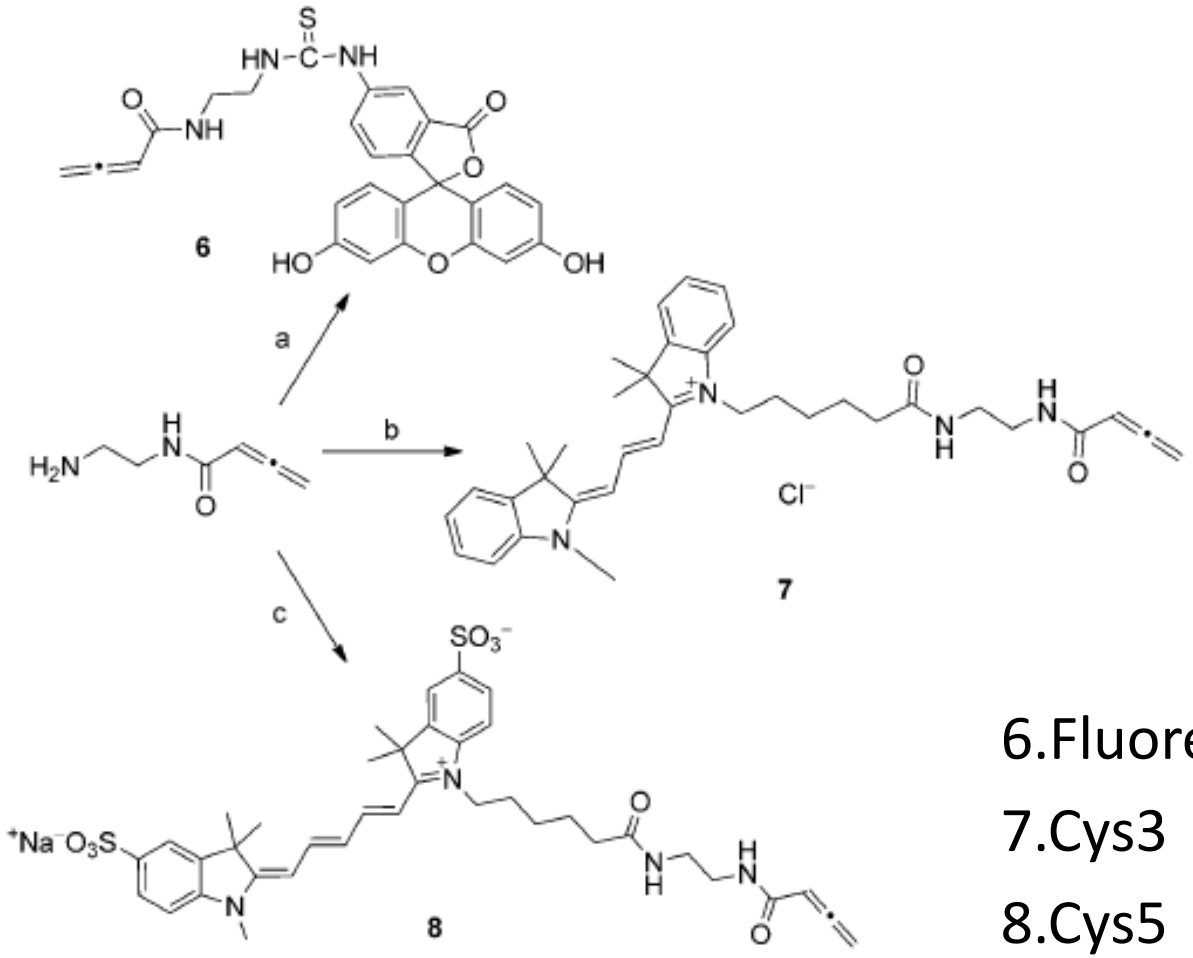
Mechanism

1,4-Michael addition



Scheme 2. Proposed mechanism for addition of thiols to allenamides.

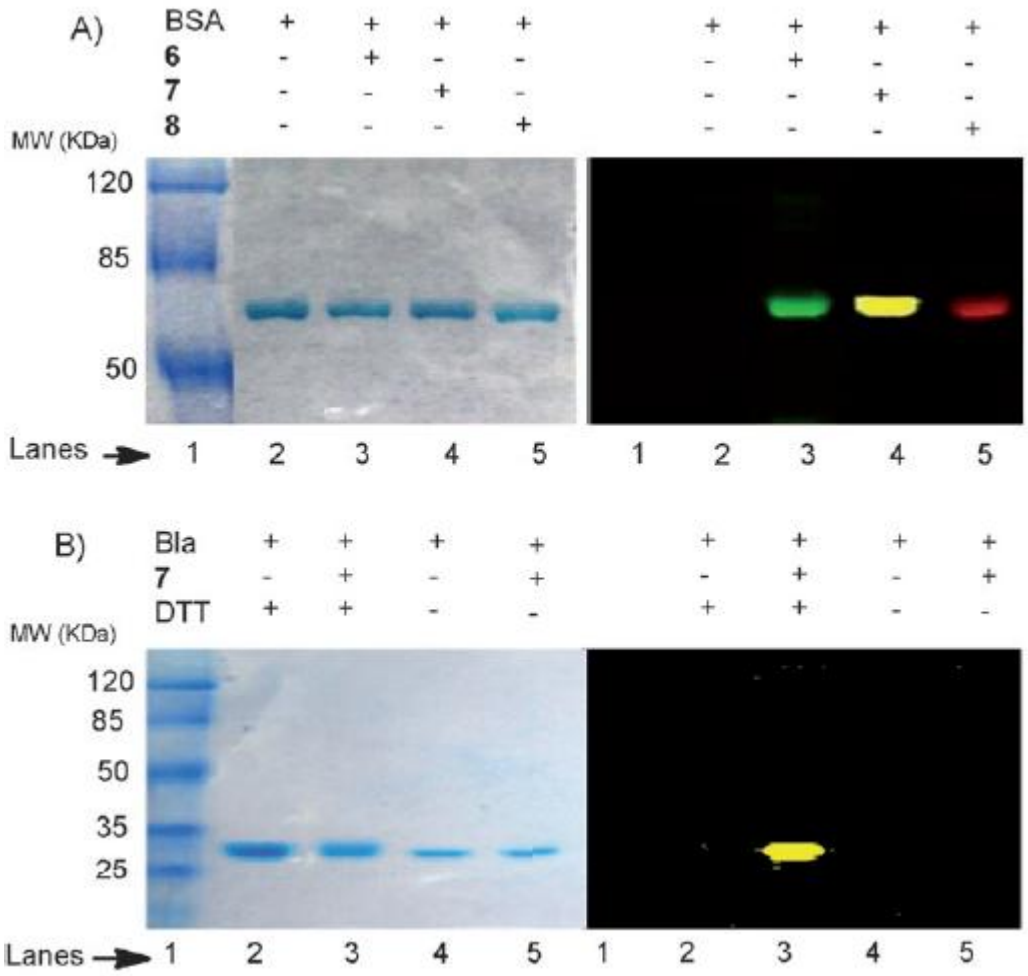
Application in selective labeling of proteins



6.Fluorescein isomer 1

7.Cys3

8.Cys5



Allenamide as an efficient handle to target cysteine residues selectively in the complex milieu of the protein environment and opens an alternative approach for imaging applications.

Summary

1. A new orthogonal handle, allenamide, to modify thiol groups in peptides and proteins selectively.
2. Irreversibility of this process can be exploited in many in vivo applications such as the inhibition of cysteine proteases.
3. Successfully label proteins with high selectivity.

Procotol： Protein chemical modification

1. A orthogonal reaction
2. Modify a kind of amino acid with high selectivity
3. Verify and text in proteins by MS and MS/MS
4. Application for a problem of life is better

Thank you !